

Supplementary Information

Tailored Polyester Nanoparticles: Post-modification with Dendritic Transporter and Targeting Units via Reductive Amination and Thiol-ene Chemistry

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Summary of Final Compounds:

Particle Type ^a	Targeting Peptides ^b	Alexa Fluor® Dye	Dendritic Molecular Transporter ^c	Compound Name ^d	Compound Class
ABD	HVGGSSV	-	-	ABD-NP-HVGGSSV (3)	NP-P
AbBD	HVGGSSV	-	-	AbBD-NP-HVGGSSV (14)	NP-P
AbBD	cHVGGSSV	594	-	AbBD-NP-cHVGGSSV-594 (8)	NP-P-dye
AbBD	cRGD	594	-	AbBD-NP-594-cRGD (10)	NP-P-dye
AbBD	-	594	MT	AbBD-NP-594-MT (6)	NP-MT-dye
AbBD	cHVGGSSV	594	MT	AbBD-NP-cHVGGSSV-594-MT(11)	NP-P-MT-dye
AbBD	cRGD	594	MT	AbBD-NP-594-cRGD-MT (12)	NP-P-MT-dye
AbBD	HVGGSSV	594	MT	AbBD-NP-HVGGSSV-594-MT (16)	NP-P-MT-dye

Summary of nanoparticle conjugates with definition of particle type depending on linear polymer precursor^a and connected targeting peptide^b: ‘c’ for capped N-terminus of peptide with HVGGSSV recognition unit via N-acetoxysuccinimide and ‘c’ for cyclic RGD. ^c Dendritic molecular transporter is abbreviated as MT, and the compound name is given in the order of the attachment ^d.

Characterization. ^1H NMR spectra were obtained from a Bruker DPX-300 or a Bruker AV-II 600 MHz spectrometer. ^{13}C NMR spectra were obtained from a Bruker AV-I 400 MHz spectrometer. Chemical shifts are reported in ppm and referenced to the corresponding residual nuclei in deuterated solvents. Gel-permeation chromatography (GPC) was carried out with a Waters chromatograph system equipped with a Waters 2414 refractive index detector, a Waters 2481 dual λ absorbance detector, a Waters 1525 binary HPLC pump, and four 5 mm Waters columns (300 mm x 7.7 mm), connected in series with increasing pore size (100, 1000, 100,000 and 1,000,000 Å respectively). All runs were performed with tetrahydrofuran (THF) as the eluent at a flow rate of 1 mL/min. For dynamic light scattering (DLS), a Malvern Nano ZS system by Malvern Instruments (Malvern Zetasizer Nanoseries, Malvern, UK) was employed at a fixed angle of 90° at 25 °C, taking the average of three measurements. The particles were diluted with toluene to a concentration, which gave the desired number of counts in order to obtain a good signal-to-noise ratio. Static light scattering was also performed on the Malvern Nano ZS to obtain the absolute weight average molecular weights of the nanoparticles. Different sample concentrations (0.25-0.67 mg/mL) were prepared by dilution of a high concentration stock solution in toluene (1 mg/mL). Data collection and calculations were managed using the Molecular Weight function in the DTS software for the Nano ZS system, which compiles the static intensity measurements, generates a standard Debye plot, and then calculates the weight

average molecular weight. Samples for transmission electron microscopy (TEM) imaging were prepared by dissolving 0.5 mg nanoparticles in 1 mL isopropanol, 0.3 mL acetonitrile and 0.2 mL toluene. The samples were sonicated for 5 min and were stained with 3 drops of 3% phosphotungstic acid. The carbon grids were prepared by slowly dipping an Ultrathin Carbon Type-A 400 Mesh Copper Grid (Ted Pella, Inc., Redding, CA) into the particle solutions three times and drying the grid at ambient temperature. A Philips CM20T transmission electron microscope operating at 200 kV in bright-field mode was used to obtain TEM micrographs of the polymeric nanoparticles. Reverse-phase high-performance liquid chromatography (RP-HPLC) was carried out with a Waters HPLC using two Delta-PakTM PrepLCTM 25 mm Columns (Waters, C18, 300Å, 25 x 100 mm each) with a PrepLCTM 25 mm Radial Compression Module. The products were eluted using a solvent gradient (solvent A = 0.05% trifluoroacetic acid (TFA)/ H₂O; solvent B = 0.05% TFA/ CH₃CN). Accurate molecular mass and purity of the peptides were determined by MALDI-MS, with α -cyano-4-hydroxycinnamic acid as the matrix, on a Perspective Biosystems Voyager-DE STR (Framingham, MA) equipped with delayed extraction technology operating in reflector mode.

Materials. Reagent chemicals were purchased from Aldrich and Acros, and used as received, unless otherwise stated. Spectra/Por[®] Dialysis membrane and SnakeSkin[®] Pleated Dialysis Tubing, regenerated cellulose, were purchased from Spectrum Laboratories Inc. and Pierce Biotechnology, respectively. Size exclusion chromatography was performed with Sephadex LH-20 from GE Healthcare Life Sciences. Molecular transporter,¹ α -allyl- δ -valerolactone, and 2-oxepane-1,5-dione² were synthesized as previously reported in the literature.

Synthesis of copolymer poly(vl-avl) (Ab). A 50 mL 3-necked round bottom flask, equipped with stir bar, was sealed with two septa and a gas inlet. The flask was evacuated and refilled with nitrogen three times. Stock solutions of 1.7 M ethanol (EtOH) in THF and 3.7×10^{-2} M tin(II) 2-ethylhexanoate ($\text{Sn}(\text{Oct})_2$) in THF were made in sealed N_2 purged flasks. Solutions of EtOH (0.32 mL, 5.41×10^{-1} mmol) and $\text{Sn}(\text{Oct})_2$ (0.30 mL, 1.12×10^{-2} mmol) were combined in the nitrogen purged 50 mL flask. After stirring the mixture for 30 min, α -allyl- δ -valerolactone (1.16 g, 8.32 mmol) and δ -valerolactone (vl, 2.50 g, 24.97 mmol) were added. The reaction vessel stirred at 105 °C for 48 h. Residual monomer and catalyst were removed by dialyzing with Spectra/Por[®] dialysis membrane (MWCO = 1000) against CH_2Cl_2 to give a golden brown polymer, **Ab** (3.24 g, 88%). $M_w = 3400$ Da, PDI = 1.16; δ_{H} (300 MHz; CDCl_3 ; Me_4Si) 5.7 (m, $\text{H}_2\text{C}=\text{CH}-$), 5.09 (m, $\text{H}_2\text{C}=\text{CH}-$), 4.09 (m, $-\text{CH}_2-\text{O}-$), 3.65 (m, $\text{CH}_3\text{CH}_2\text{O}-$), 2.35 (m, vl - $\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{O}-$, avl $\text{H}_2\text{C}=\text{CHCH}_2\text{CH}-$, $\text{H}_2\text{C}=\text{CHCH}_2\text{CH}-$), 1.68 (m, avl & vl $-\text{CHCH}_2\text{CH}_2-$), 1.25 (t, $\text{CH}_3\text{CH}_2\text{O}-$); δ_{C} (400 MHz; CDCl_3) 174.6 (avl $-\text{C}(\text{O})-$), 172.7 (vl $-\text{C}(\text{O})-$), 134.6 ($\text{H}_2\text{C}=\text{CH}-$), 116.4 ($\text{H}_2\text{C}=\text{CH}-$), 63.3, 44.3, 35.9, 33.1, 27.5, 25.9, 23.6, 20.9.

Oxidation of poly(vl-evl) (AB). In a 200 mL round bottom flask, equipped with stir bar, poly(vl-avl) (2.74 g, 6.12 mmol) was dissolved in 37 mL of CH_2Cl_2 . To this solution, 3-chloroperoxybenzoic acid (2.09 g, 12.11 mmol) was added slowly. The mixture was stirred for 72 h at room temperature and then concentrated via rotary evaporator. The crude product was dissolved in a minimal amount of THF (5 mL) and poured into a round-bottomed flask containing 1L diethyl ether. The solution was kept overnight at 0 °C and a white solid was obtained. The solution was decanted off and the solid was dried *in vacuo* to obtain poly(vl-evl),

AB (1.95 g, 71%). δ_{H} (300 MHz; CDCl_3 ; Me_4Si) 4.09 (m, $-\text{CH}_2-\text{O}-$), 3.65 (m, $\text{CH}_3\text{CH}_2\text{O}-$), 2.96 (m, $-\text{CH}(\text{O})\text{CH}_2-$), 2.75 (m, $-\text{CH}(\text{O})\text{CH}_2-$), 2.47 (m, $-\text{CH}(\text{O})\text{CH}_2-$), 2.35 (m, vl $-\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{O}-$, evl $-\text{CHCH}_2\text{CH}-$, $-\text{CHCH}_2\text{CH}-$), 1.68 (m, evl & vl $-\text{CHCH}_2\text{CH}_2-$), 1.25 (t, $\text{CH}_3\text{CH}_2\text{O}-$).

General procedure for drop in method synthesis of nanoparticles from poly(vl-evl) (AB).

In a 100 mL three-necked round bottom flask equipped with stir bar, condenser and septa, 2,2'-(ethylenedioxy)bisethylamine (34.1 μL , 2.32×10^{-4} mol), 28.7 mL CH_2Cl_2 . A solution of poly(vl-evl), **AB**, (0.14 g, $M_{\text{w}} = 3400$ Da, PDI = 1.16) in 0.19 mL CH_2Cl_2 was added dropwise via a peristaltic pump at 13 mL/min with vigorous stirring. The mixture was heated at 44 °C for 12 h. Residual diamine was removed by dialyzing with SnakeSkin[®] Pleated Dialysis Tubing (MWCO = 10,000) against dichloromethane. DLS: $D_{\text{H}} = 255.7 \pm 22.5$ nm. δ_{H} (300MHz; CDCl_3 ; Me_4Si) The significant change is the disappearance of the epoxide protons at 2.94, 2.75 and 2.47 ppm and the appearance of signals at 3.64 and 2.97 ppm corresponding to the protons neighboring the secondary amine of the PEG linker after cross-linking. All other aspects of the spectrum are similar.

General procedure for the one pot synthesis of nanoparticles from poly(vl-evl) (AB). In a 100 mL three-necked round bottom flask equipped with stir bar, condenser and septa, 2,2'-(ethylenedioxy)bisethylamine (34.1 μL , 2.32×10^{-4} mol), 28.7 mL CH_2Cl_2 and a solution of poly(vl-evl), **AB**, (0.14 g, $M_{\text{w}} = 3400$ Da, PDI = 1.16) in 0.19 mL CH_2Cl_2 were added. The mixture was heated at 44 °C for 12 h. Residual diamine was removed by dialyzing with SnakeSkin[®] Pleated Dialysis Tubing (MWCO = 10,000) against dichloromethane. DLS: $D_{\text{H}} =$

272.3 \pm 23.3 nm. δ_H (300MHz; CDCl₃; Me₄Si) The significant change is the disappearance of the epoxide protons at 2.94, 2.75 and 2.47 ppm and the appearance of signals at 3.64 and 2.97 ppm corresponding to the protons neighboring the secondary amine of the PEG linker after cross-linking. All other aspects of the spectrum are similar.

Synthesis of copolymer poly(vl-avl-opd) (AbD). To a 25 mL 3-necked round bottom flask, equipped with stir bar, gas inlet and 2 rubber septa, 2-oxepane-1,5-dione (0.70 g, 5.46 mmol) was added. The round bottom flask was purged with argon. After purging for 30 min, dry toluene (4 mL) was added. The mixture stirred in an oil bath at 80 °C to dissolve the monomer. Upon dissolving, Sn(Oct)₂ (11.1 mg, 27.3 μ mol) in 0.5 mL dry toluene, absolute ethanol (20.5 mg, 440 μ mol), α -allyl- δ -valerolactone (1.15 g, 8.19 mmol) and δ -valerolactone (1.37 g, 13.7 mmol) were then added to the reactor and the mixture was heated for 48 h at 105 °C. Residual monomer and catalyst were removed by dialyzing with Spectra/Por[®] dialysis membrane (MWCO = 1000) against CH₂Cl₂ to give a golden brown polymer, **AbD** (2.70 g, 85%). M_w = 3287 Da, PDI = 1.17; δ_H (300 MHz; CDCl₃; Me₄Si) 5.72 (m, H₂C=CH-), 5.06 (m, H₂C=CH-), 4.34 (m, -CH₂CH₂C(O)CH₂CH₂O-), 4.08 (m, -CH₂O-), 3.67 (m, -OCH₂CH₃), 2.78 (m, opd -OC(O)CH₂CH₂C(O)CH₂-), 2.58 (m, opd -OC(O)CH₂CH₂C(O)CH₂-), 2.34 (m, vl -CH₂CH₂C(O)O-, avl H₂C=CHCH₂CH-, H₂C=CHCH₂CH-), 1.66 (m, avl & vl -CHCH₂CH₂-), 1.25 (t, -CH₂CH₃); δ_C (400 MHz; CDCl₃) 204.9, 175.2, 173.7, 173.2, 135.0, 117.0, 63.9, 44.8, 36.4, 33.6, 28.0, 26.3, 21.3.

Synthesis of poly(vl-evl-opd) (ABD). To a solution of **AbD** (2.70 g, 4.67 mmol) in CH_2Cl_2 (37 mL), 3-chloroperoxybenzoic acid (1.46 g, 8.48 mmol) was added. The mixture stirred for 72 h at room temperature and then concentrated via rotary evaporator. The crude product was dissolved in a minimal amount of tetrahydrofuran (THF) (5 mL) and dropped into a round bottom flask containing 1L diethyl ether. The solution was kept overnight at 0 °C and a white solid was obtained. The solution was decanted off and the solid was dried *in vacuo* to obtain **ABD** (1.95 g, 72%). $M_w = 3392$ Da, PDI = 1.19. δ_H (300 MHz; CDCl_3 ; Me_4Si) 4.34 (m, - $\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{CH}_2\text{CH}_2\text{O}-$), 4.08 (m, - $\text{CH}_2\text{O}-$), 3.67 (m, - OCH_2CH_3), 2.96 (m, - $\text{CH}(\text{O})\text{CH}_2-$), 2.78 (m, - $\text{CH}(\text{O})\text{CH}_2-$, opd - $\text{OC}(\text{O})\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{CH}_2-$), 2.58 (m, opd - $\text{OC}(\text{O})\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{CH}_2-$), 2.47 (m, - $\text{CH}(\text{O})\text{CH}_2-$), 2.34 (m, vl - $\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{O}-$, evl - $\text{CHCH}_2\text{CH}-$, - $\text{CHCH}_2\text{CH}-$), 1.66 (m, evl & vl, - $\text{CHCH}_2\text{CH}_2-$), 1.25 (t, - CH_2CH_3)

Nanoparticle formation from poly(vl-evl-opd) (ABD). A solution of **ABD** (0.11 g, $M_w = 3392$ Da, PDI = 1.19) dissolved in CH_2Cl_2 (0.26 mL) was added to a solution of 2,2'-(ethylenedioxy)diethylamine (76.4 μL , 0.52 μmol) in CH_2Cl_2 (40.3 mL). The mixture was heated for 12 h at 44 °C with vigorous stirring. Residual diamine was removed by dialyzing with SnakeSkin[®] Pleated Dialysis Tubing ($MWCO = 10,000$) against dichloromethane to yield nanoparticles (0.17 g, 91%). DLS: $D_H = 118.3 \pm 9.6$ nm. SLS: $M_w = 323,000$. δ_H (300 MHz; CDCl_3 ; Me_4Si) The significant change is the disappearance of the epoxide protons at 2.94, 2.75 and 2.47 ppm and the appearance of signals at 3.54 and 2.97 ppm corresponding to the protons neighboring the secondary amine of the PEG linker after cross-linking. All other aspects of the spectrum are similar.

N-Boc-ethylenediamine (NBED) conjugated ABD nanoparticles. To a solution of **ABD** nanoparticles (20 mg, 0.06 μmol) in THF (2 mL), N-acetoxysuccinimide (0.02 g, 0.13 mmol) was added. The reaction mixture stirred for 3 h. Residual N-acetoxysuccinimide was removed by dialyzing with SnakeSkin[®] Pleated Dialysis Tubing (MWCO = 10,000) against THF. Once the product was concentrated and dried, the nanoparticles (18 mg, 0.05 μmol) were dissolved in a mixture of CH_2Cl_2 and CH_3OH (1:1, v/v, 2 mL). To this solution, N-Boc-ethylenediamine (4.6 μL of 1.59 M NBED in CH_3OH) and NaCNBH_3 (21.8 μL of 1.0 M NaCNBH_3 in THF) were added. The reaction mixture stirred for 12 h at room temperature and then was purified by dialyzing with SnakeSkin[®] Pleated Dialysis Tubing (MWCO = 10,000) against 1:1 $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ to yield NBED conjugated nanoparticles (18 mg, 88%). DLS: $D_H = 119.5 \pm 10.3$ nm; original particle $D_H = 118.3 \pm 9.6$ nm. δ_H (300 MHz; CDCl_3 ; Me_4Si) The significant change is the appearance of the peak at 1.43 ppm due to the Boc protecting group. All other aspects of the spectrum are similar to that of the **ABD** nanoparticles.

General procedures for the synthesis of HVGGSSV peptide (1). The HVGGSSV peptide was synthesized by solid-phase peptide synthesis using standard Fmoc chemistry on a Model 90 Peptide Synthesizer (Advanced ChemTech).

General procedure: Attachment of N-Fmoc amino acids to resin. After swelling with dichloromethane (20 mL) for 20 min, H-val-2-Cl-Trt resin (0.20 g, 1.03 mmol/g, 0.21 mmol surface amino acids) was treated with a solution of Fmoc-protected amino acids (4.4 equiv, 0.9 mmol) in dimethylformamide (DMF) (9 mL). The amino acids were attached to the resin using double coupling with a solution (9 mL) consisting of N-hydroxybenzotriazole monohydrate

(HOBt) (0.9 mmol, 0.14 g) *o*-(benzotriazole-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (0.9 mmol, 0.34 g), *N,N'*-diisopropylethylamine (DIPEA) (1.8 mmol, 0.31 mL) in 9 mL DMF. The reaction mixture was shaken for 60 min and washed with DMF (4 x 10 mL), methanol (4 x 10 mL) and DMF (4 x 10 mL). The end of the coupling was controlled by the Ninhydrin test. A 20% (v/v) piperidine in DMF solution was used to deprotect the Fmoc groups. The amino acids were attached to the resin in the following sequence: Ser, Ser, Gly, Gly, Val, His, Asn, Gly, Gly, Gly, Cys, and Gly.

General procedure: Cleavage from resin. The resin was treated with Reagent R, a solution of TFA, thioanisole, anisole, and ethanedithiol (90:5:3:2, 6 mL), for 4 h. After removal of the resin by filtration, the filtrate was concentrated to precipitate the peptide with cold diethyl ether. Crude peptides were purified by RP-HPLC and lyophilized. Peptide identity was confirmed by MS. m/z (MALDI) 1088.1 (M^+ , 98%).

HVGGSSV conjugated ABD nanoparticles (3). To a solution of **ABD** nanoparticles (20.0 mg, 0.06 μ mol) in THF (2 mL), *N*-acetoxysuccinimide (3 mg, 18.1 μ mol) was added. The reaction mixture stirred for 3 h. Residual *N*-acetoxysuccinimide was removed by dialyzing with SnakeSkin[®] Pleated Dialysis Tubing (MWCO = 10,000) against 1:1 THF/CH₃OH to give amine capped **ABD** nanoparticles, **2**. To a solution of **2** (0.0174 g, 0.05 μ mol, in 3 mL THF), **1** (3.5 mg, 3.18 μ mol) dissolved in DMSO (2 mL) and NaCNBH₃ (6.36 μ L 1.0 M NaCNBH₃ in THF) were added. The reaction mixture stirred for 12 h at room temperature. The reaction mixture was purified by dialyzing with SnakeSkin[®] Pleated Dialysis Tubing (MWCO = 10,000) against 1:1 THF/CH₃CN to yield **3** (19 mg, 88%). DLS: $D_H = 120.5 \pm 10.2$ nm; original particle $D_H = 118.3$

± 9.6 nm. SLS: $M_w = 362,000$; original particle $M_w = 323,000$. δ_H (600 MHz; $(CD_3)_2SO$) The significant change is the appearance of the following peaks: 8.26-7.87, 7.42, 6.90, 4.39, 3.71 and 2.01 ppm due to the attachment of the peptide. All other aspects of the spectrum are similar to that of the **ABD** nanoparticles.

Synthesis of poly(vl-avl-evl-opd) (AbBD). To a solution of **AbD** (1.70 g, 1.56 mmol) in CH_2Cl_2 (30 mL), 3-chloroperoxybenzoic acid (0.22 g, 1.28 mmol) was added. The mixture stirred for 72 h at room temperature and then was concentrated via rotary evaporator. The crude product was dissolved in a minimal amount of THF (5 mL) and poured into a round bottom flask containing 1L diethyl ether. The solution was kept overnight at 0 °C and a white solid was obtained. The solution was decanted off and the solid was dried *in vacuo* to obtain **AbBD** (1.2 g, 71%). $M_w = 3356$ Da, PDI = 1.18. δ_H (300 MHz; $CDCl_3$; Me_4Si) 5.72 (m, $H_2C=CH-$), 5.06 (m, $H_2C=CH-$), 4.34 (m, $-CH_2CH_2C(O)CH_2CH_2O-$), 4.08 (m, $-CH_2O-$), 3.67 (m, $-OCH_2CH_3$), 2.96 (m, $-CH(O)CH_2-$), 2.78 (m, $-CH(O)CH_2-$, opd $-OC(O)CH_2CH_2C(O)CH_2-$), 2.58 (m, opd $-OC(O)CH_2CH_2C(O)CH_2-$), 2.47 (m, $-CH(O)CH_2-$), 2.34 (m, vl $-CH_2CH_2C(O)O-$, avl $H_2C=CHCH_2CH-$, $H_2C=CHCH_2CH-$), 1.66 (m, avl & vl $-CHCH_2CH_2-$), 1.25 (t, $-CH_2CH_3$).

General procedure for drop in method synthesis of nanoparticles from AbBD. A solution of **AbBD** (0.21 g, $M_w = 3356$ Da, PDI = 1.18) dissolved in CH_2Cl_2 (0.39 mL) was added dropwise via a peristaltic pump at 13 mL/min with vigorous stirring to a solution of 2,2'-(ethylenedioxy)diethylamine (42.6 μL , 0.29 mmol) in CH_2Cl_2 (60 mL) at 44 °C. The reaction mixture was heated for 12 h. Residual diamine was removed by dialyzing with SnakeSkin[®]

Pleated Dialysis Tubing (MWCO = 10,000) against dichloromethane to obtain nanoparticles (0.24 g, 96%). DLS: $D_H = 123.4 \pm 9.22$ nm. SLS: $M_w = 345,000$. δ_H (300 MHz; $CDCl_3$; Me_4Si) The significant change is the disappearance of the epoxide protons at 2.96, 2.75 and 2.47 ppm and the appearance of signals at 3.56 and 2.98 ppm corresponding to the protons neighboring the secondary amine of the PEG linker after cross-linking. All other aspects of the spectrum are similar to that of **AbBD**.

General procedure for one pot synthesis of nanoparticles from AbBD. To a solution of 2,2'-(ethylenedioxy)diethylamine (26.2 μ L, 0.18 mmol) in CH_2Cl_2 (34.6 mL), a solution of **AbBD** (0.13 g, $M_w = 3356$ Da, PDI = 1.18) in CH_2Cl_2 (0.24 mL) was added. The mixture was heated at 44 °C for 12 h. Residual diamine was removed by dialyzing with SnakeSkin[®] Pleated Dialysis Tubing (MWCO = 10,000) against CH_2Cl_2 to obtain nanoparticles (0.15 g, 94%). DLS: $D_H = 126.6 \pm 9.3$ nm. SLS: $M_w = 350,000$. δ_H (300 MHz; $CDCl_3$; Me_4Si) The significant change is the disappearance of the epoxide protons at 2.94, 2.75 and 2.47 ppm and the appearance of signals at 3.54 and 2.97 ppm corresponding to the protons neighboring the secondary amine of the PEG linker after cross-linking. All other aspects of the spectrum are similar to that of **AbBD**.

General procedure for the attachment of benzyl mercaptan to AbBD nanoparticles. To a solution of **AbBD** nanoparticles (15 mg, 0.04 μ mol) in toluene (0.5 mL), benzyl mercaptan (3.5 μ L, 29 μ mol) was added. The reaction mixture was heated for 72 h at 35 °C. The remaining toluene was removed *in vacuo* and residual benzyl mercaptan was removed by dialyzing with SnakeSkin[®] Pleated Dialysis Tubing (MWCO = 10,000) against CH_2Cl_2 . δ (300 MHz; $CDCl_3$;

Me₄Si) The significant change is the reduction of the allyl protons at 5.72 and 5.06 ppm and the appearance of signals at 3.73 and 7.30 ppm corresponding to the methylene and benzene protons respectively of the attached benzyl mercaptan. All other aspects of the spectrum are similar to that of **AbBD** nanoparticles.

Molecular transporter (MT) dithiol cleavage (5) (contribution of Sharon Hamilton). To a solution of LL-MT (15 mg, 4.56 μmol) in CH₃OH (0.4 mL), a solution of *D,L*-dithiothreitol in CH₃OH (0.2 mL) was added. The reaction mixture stirred for 3 h at room temperature. Residual dithiothreitol was removed by purification with Sephadex LH-20. The product was immediately attached to **ABbD** nanoparticles.

Model reaction of attachment of MT to AbBD nanoparticles. To a solution of **AbBD** nanoparticles (15 mg, 0.04 μmol) in CH₃OH (0.2 mL), **5** (11 mg, 3.35 μmol) in CH₃OH (0.4 mL) was added. The reaction mixture was heated for 72 h at 37 °C. Residual **5** was removed by dialyzing with SnakeSkin[®] Pleated Dialysis Tubing (MWCO = 10,000) against methanol. To obtain MT conjugated nanoparticles (31.3 mg, 89%). DLS: D_H = 128.9 ± 10.2 nm; original particle D_H = 126.6 ± 9.3 nm. δ_H (300 MHz; CD₃OD) The significant change is the reduction of the allyl protons at 5.72 and 5.06 ppm and the appearance of signals at 2.20-1.98 (m, CH₂), 1.57 (m, CH₂) and 1.39 (m, CH₂) ppm due to the dendritic backbone of the MT. All other aspects of the spectrum are similar to that of **AbBD** nanoparticles.

Alexa Fluor[®] 594 conjugated AbBD nanoparticles (4). To a solution of **AbBD** nanoparticles (0.021 g, 0.06 μmol) in dry THF (1.5 mL), Alexa Fluor[®] 594 (0.14 mL of 10

mg/mL Alexa Fluor® 594 in DMF, 1.7 μ mol) was added. The reaction mixture stirred for 24 h at room temperature. Residual Alexa Fluor® 594 was removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against CH₃OH to obtain **4** (15.2 mg, 88%). δ_{H} (300 MHz; CD₃OD) The significant change is the appearance of the following peaks due to Alexa Fluor® 594: 7.92, 7.41, 4.48, 3.62, and 1.24 ppm. δ_{H} (600 MHz; (CD₃)₂SO) The significant change is the appearance of the following peaks due to Alexa Fluor® 594: 7.92, 7.47, 4.44, 3.58, and 1.25 ppm. All other aspects of the spectrum are similar to that of **AbBD** nanoparticles.

Attachment of MT to Alexa Fluor® 594 conjugated AbBD nanoparticles, AbBD-NP-594-MT (6). To a solution of **4** (8 mg, 0.89 μ mol) in CH₃OH (0.2 mL), **5** (7.5 mg, 2.27 μ mol) in CH₃OH (0.4 mL) was added. The reaction mixture was heated for 72 h at 37 °C. Residual **5** was removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against CH₃OH. Yield: 10.0 mg (91%). DLS: $D_{\text{H}} = 129.4 \pm 9.8$ nm; original particle $D_{\text{H}} = 126.6 \pm 9.3$ nm. SLS: $M_{\text{w}} = 445,000$; original particle $M_{\text{w}} = 350,000$. δ_{H} (300 MHz; CD₃OD) The significant change is the reduction of the allyl protons at 5.72 and 5.06 ppm and the appearance of signals at 2.20-1.98 (m, CH₂), 1.57 (m, CH₂) and 1.39 (m, CH₂) ppm due to the dendritic backbone of the MT. All other aspects of the spectrum are similar to that of **4**.

N-acetoxysuccinimide conjugated HVGGSSV peptide, cHVGGSSV (7). To a solution of **1** (29.4 mg, 2.7×10^{-5} mol) dissolved in CH₃CN (3 mL), N-acetoxysuccinimide (0.42 g, 2.7×10^{-3} mol) was added. The reaction mixture stirred for 3 h at room temperature. After removal of the solvent under reduced pressure, the crude product was purified by RP-HPLC. m/z (MALDI) 1175 (M^+ , 97%).

Capped HVGGSSV conjugated Alexa Fluor® 594-AbBD nanoparticles, AbBD-NP-cHVGGSSV-594 (8). To a solution of **AbBD** nanoparticles (0.021 g, 0.06 μmol) in dimethylsulfoxide (0.7 mL), **7** (6.4 mg, 5.46 μmol) was added. The reaction mixture was heated for 72 h at 33 °C. To this solution, Alexa Fluor® 594 (0.14 mL of 10 mg/mL Alexa Fluor® 594 in DMF, 1.7 μmol) was added. Residual Alexa Fluor® 594 and peptide were removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against 1:1 $\text{CH}_3\text{OH}/\text{CH}_3\text{CN}$ to obtain **8** (20.1 mg, 80%). DLS: $D_H = 128.9 \pm 10.9$ nm; original particle $D_H = 126.6 \pm 9.3$ nm. SLS: $M_w = 404,000$; original particle $M_w = 350,000$. δ_H (600 MHz; $(\text{CD}_3)_2\text{SO}$) The significant change is the reduction of the allyl protons at 5.72 and 4.97 ppm and the appearance of the following sets of significant signals: 8.21, 7.33, 4.35, 3.73 and 0.80 ppm due to the peptide, and 7.92, 7.48, 7.27, 4.44, and 1.25 ppm due to the Alexa Fluor® 594. All other aspects of the spectrum are similar to that of **AbBD** nanoparticles.

Attachment of MT to cHVGGSSV conjugated Alexa Fluor® 594-AbBD nanoparticles, AbBD-NP-cHVGGSSV-594-MT (11). To a solution of **8** (6 mg, 0.02 μmol) in DMSO (0.1 mL), **5** (2 mg, 0.88 μmol) in CH_3OH (0.3 mL) was added. The reaction mixture was heated for 48 h at 33°C. Residual **5** was removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against 1:1 $\text{CH}_3\text{OH}/\text{CH}_3\text{CN}$ to yield **11** (7.4 mg, 93%). DLS: $D_H = 130.7 \pm 9.4$ nm; original particle $D_H = 126.6 \pm 9.3$ nm. SLS: $M_w = 473,000$; original particle $M_w = 350,000$. δ_H (600 MHz; $(\text{CD}_3)_2\text{SO}$) The significant change is the reduction of the allyl protons at 5.72 and 4.97 ppm and the appearance of signals at 3.06 (m, CH_2), 2.96 (m, CH_2), 1.97 (m, CH_2),

1.77 (m, CH₂), 1.41 (m, CH₂), 1.35 (m, CH₂), 0.95 and 0.8 (m, CH₂) ppm due to the dendritic backbone of the MT. All other aspects of the spectrum are similar to that of **8**.

Synthesis of cyclic RGD, cRGD (9) (contribution of Teresa Croce). The RGD peptide was synthesized by solid-phase peptide synthesis using standard Fmoc chemistry on a Model 90 Peptide Synthesizer (Advanced ChemTech).

Synthesis of Linear RGD. After swelling with dichloromethane (20 mL), Fmoc-Cys-2-Cl-Trt resin (0.20 g, 0.9 mmol/g, 0.18 mmol surface amino acids) was deprotected with a 20% (v/v) piperidine in DMF solution and treated with a solution of Fmoc-protected amino acid (4.4 equiv, 0.9 mmol) in dimethylformamide (DMF) (9 mL). The amino acids were attached to the resin using double coupling with a solution (9 mL) consisting of N-hydroxybenzotriazole monohydrate (0.9 mmol, 0.14 g) *o*-(benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate (0.9 mmol, 0.34 g), N,N'-diisopropylethylamine (1.8 mmol, 0.31 mL) in 9 mL DMF. The reaction mixture was shaken for 60 min and washed with DMF (4 x 10 mL), methanol (4 x 10 mL) and DMF (4 x 10 mL). A 20% (v/v) piperidine in DMF solution was used to deprotect the Fmoc groups. An amino-hexyl spacer was coupled to the cystine on the resin, followed by glutamic acid, aspartic acid, glycine, arginine, phenylalanine, and finally lysine.

Cyclization of RGD. The peptide was cyclized by utilizing an ODmab group, which allows for the selective deprotection carboxylic acid side chain of the glutamic acid, which can then be coupled to the N-terminus. The ODmab was deprotected using 2% v/v hydrazine monohydrate/DMF added to the resin and shaken for 7 min. Next it was washed with 20 mL of DMF followed by 10 mL of a 5% v/v DIPEA/DMF solution which was allowed to shake for 10

min. Carboxy activation was achieved through the use of N,N'-dicyclohexylcarboimide (DCC) (44.6 mg, 0.22 mmol) and hydroxybenzotriazole (HOBt) (29.2 mg, 0.22 mmol) which was added to 10 mL of DMF and then added to the resin and allowed to shake for 18 h.

General procedure: Cleavage from resin. The resin was treated with Reagent R, a solution of TFA, thioanisole, anisole, and ethanedithiol (90:5:3:2, 6 mL), for 3 h. After removal of the resin by filtration, the filtrate was concentrated to precipitate the peptide with cold diethyl ether. The crude peptide was collected by centrifugation, purified by RP-HPLC and lyophilized. Peptide identity was confirmed by MS. m/z (MALDI) 946.7 (M^+ , 98%).

Attachment of cRGD to Alexa Fluor® 594 conjugated AbBD nanoparticles, AbBD-NP-594-cRGD (10). To a solution of AbBD nanoparticles (23.0 mg, 0.07 μ mol) in THF (2.3 mL), Alexa Fluor® 594 (0.15 mL of 10 mg/mL Alexa Fluor® 594 in DMF, 1.83 μ mol) was added. After stirring the reaction mixture for 24 h at room temperature, the solvent was removed via rotary evaporator. To the Alexa Fluor® 594 conjugated nanoparticles, methanol (0.35 mL) and **9** (5.7 mg, 6.0 μ mol), dissolved in DMSO (0.35 mL), were added. The reaction mixture was heated for 72 h at 33 °C. Residual Alexa Fluor® 594 and peptide were removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against 1:1 CH₃OH/CH₃CN to obtain **10** (22.0 mg, 81%). DLS: $D_H = 129.8 \pm 9.6$ nm; original particle $D_H = 126.6 \pm 9.3$ nm. SLS: $M_w = 394,000$; original particle $M_w = 350,000$. δ_H (600 MHz; (CD₃)₂SO) The significant change is the reduction of the allyl protons at 5.72 and 4.97 ppm and the appearance of the following sets of significant signals: 7.82, 7.29, 6.05, 4.37, and 1.66 ppm due to cRGD, and 7.91, 7.52, 4.44, and

1.23 ppm due to the Alexa Fluor® 594. All other aspects of the spectrum are similar to that of **AbBD** nanoparticles.

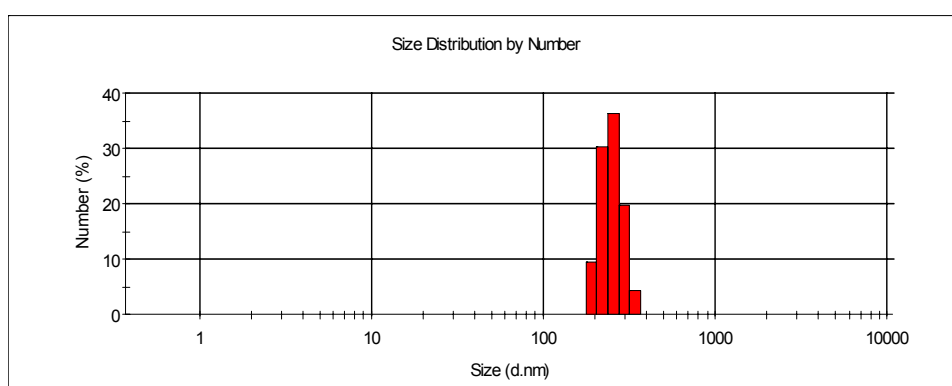
Attachment of MT to cRGD conjugated Alexa Fluor® 594-AbBD nanoparticles, AbBD-NP-594-cRGD-MT (12). To a solution of **10** (7.8 mg, 0.02 μ mol) in DMSO (0.1 mL), **5** (1.4 mg, 0.67 μ mol) in CH₃OH (0.3 mL) was added. The reaction mixture was heated for 48 h at 33°C. Residual **5** was removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against 1:1 CH₃OH/CH₃CN to yield **12** (7.6 mg, 83%). DLS: $D_H = 131.9 \pm 10.6$ nm; original particle $D_H = 126.6 \pm 9.3$ nm. SLS: $M_w = 461,000$; original particle $M_w = 350,000$. δ_H (600 MHz; (CD₃)₂SO) The significant change is the reduction of the allyl protons at 5.72 and 4.97 ppm and the appearance of signals at 3.04 (m, CH₂), 2.98 (m, CH₂), 1.98 (m, CH₂), 1.75 (m, CH₂), 1.41 (m, CH₂), 1.35 (m, CH₂) 0.95 and 0.81 (m, CH₂) ppm due to the dendritic backbone of the MT. All other aspects of the spectrum are similar to that of **10**.

HVGGSSV conjugated AbBD nanoparticles, AbBD-NP-HVGGSSV (14). To a solution of **AbBD** nanoparticles (50.0 mg, 0.14 μ mol) in THF (2 mL), N-acetoxysuccinimide (7 mg, 44.5 μ mol) was added. The reaction mixture stirred for 3 h. Residual N-acetoxysuccinimide was removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against 1:1 THF/CH₃OH to give amine capped **AbBD** nanoparticles, **13**. To a solution of **13** (50.0 mg, 0.14 μ mol, in 3 mL THF), **1** (9.3 mg, 8.57 μ mol) dissolved in DMSO (2 mL) and NaCNBH₃ (17.1 μ L 1.0 M NaCNBH₃ in THF) were added. The reaction mixture stirred for 12 h at room temperature. The reaction mixture was purified by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against 1:1 THF/CH₃CN to obtain **14** (43.2 mg, 83%). DLS: $D_H = 129.7 \pm$

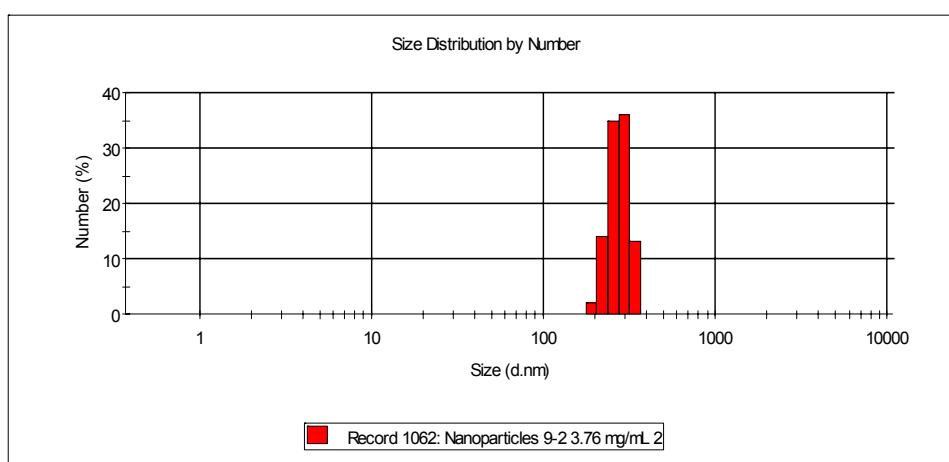
9.5 nm; original particle $D_H = 126.6 \pm 9.3$ nm. SLS: $M_w = 391,000$; original particle $M_w = 350,000$. δ_H (600 MHz; $(CD_3)_2SO$) The significant change is the appearance of the following peaks: 8.21, 7.85, 4.40, 3.73, 2.01 and 0.80 ppm due to the peptide. All other aspects of the spectrum are similar to that of **AbBD** nanoparticles.

Thiolated Alexa Fluor® 594 (15). To a solution of Alexa Fluor® 594 (0.2 mL of 10 mg/mL Alexa Fluor® 594 in DMF, 2.4 μ mol), cysteamine (68.4 μ L of 2.5 mg/mL cysteamine in DMSO, 2.2 μ mol) was added. The reaction mixture stirred for 3 h at room temperature. The product was immediately attached to **14**.

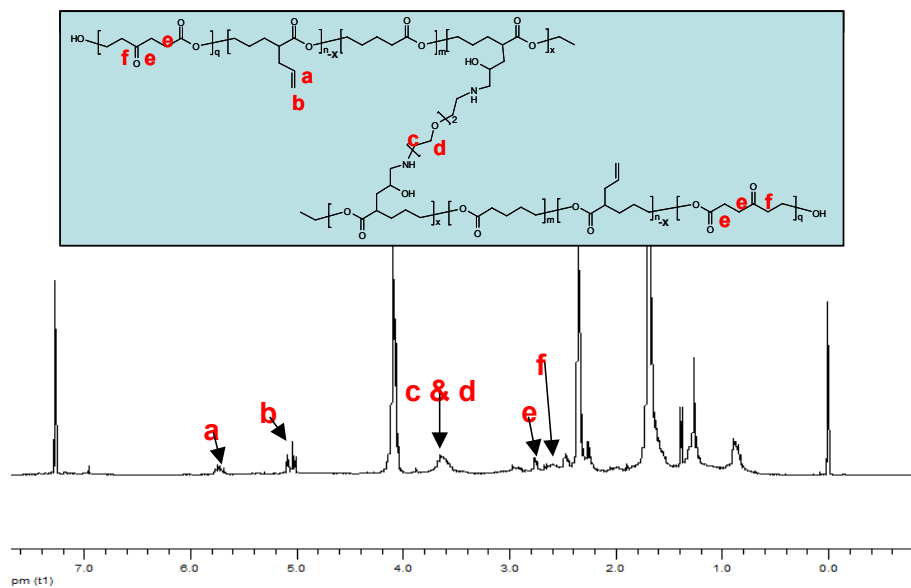
Attachment of MT to HVGGSSV conjugated Alexa Fluor® 594-AbBD nanoparticles, AbBD-NP-HVGGSSV-594-MT (16). To a solution of **14** (16 mg, 0.04 μ mol) in DMSO (0.2 mL), **15** (2 mg, 1.95 μ mol) in DMSO (0.2 mL) and **5** (2.7 mg, 1.2 μ mol) in CH_3OH (0.4 mL) were added. The reaction mixture was heated for 48 h at 33°C. Residual **5** and **15** were removed by dialyzing with SnakeSkin® Pleated Dialysis Tubing (MWCO = 10,000) against CH_3OH . Yield: 18.5 mg (86%). DLS: $D_H = 132.1 \pm 9.3$ nm; original particle $D_H = 126.6 \pm 9.3$ nm. SLS: $M_w = 475,000$; original particle $M_w = 350,000$. δ_H (600 MHz; $(CD_3)_2SO$) The significant change is the reduction of the allyl protons at 5.72 and 4.97 ppm and the appearance of the following sets of significant signals: 3.08, 2.99, 1.97, 1.79, 1.43, 1.34, 0.95 and 0.80 ppm due to the dendritic backbone of the MT, and 7.88, 7.51, 7.26, 4.52, 4.44, and 1.24 ppm due to the Alexa Fluor® 594. All other aspects of the spectrum are similar to that of **14**.



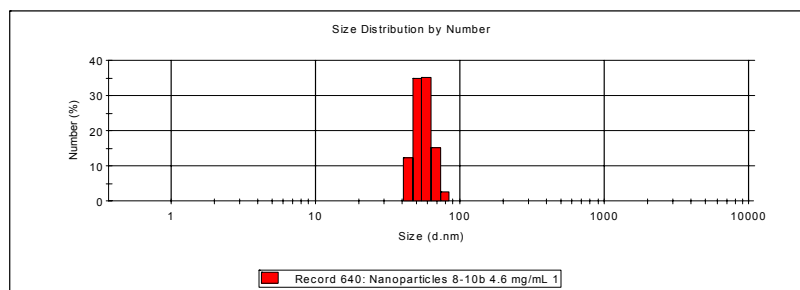
DLS size distribution profile for 255.7 ± 22.5 nm **AB** particles formed by **drop-in method**.



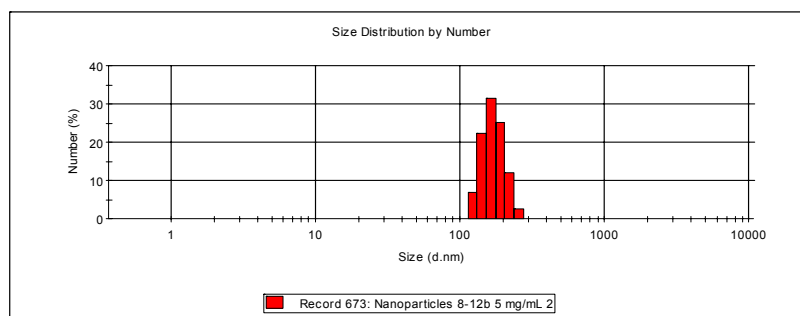
DLS size distribution profile for 272.3 ± 23.3 nm **AB** particles formed by **one-pot technique**.



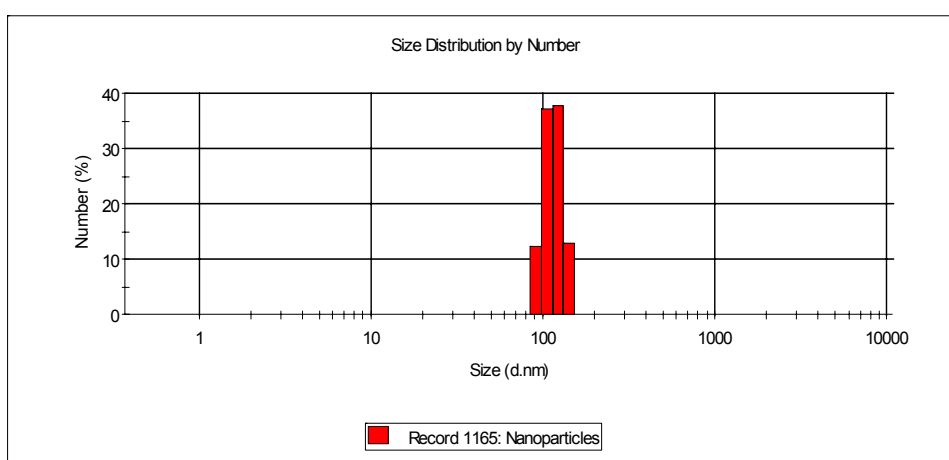
2 amines / 1 epoxide
49.32 ± 4.5 nm



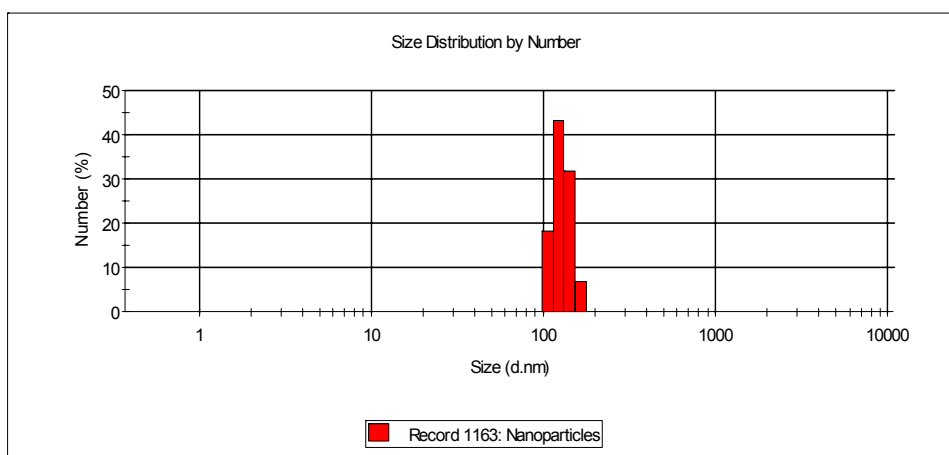
3 amines / 1 epoxide
126.6 ± 11.0 nm



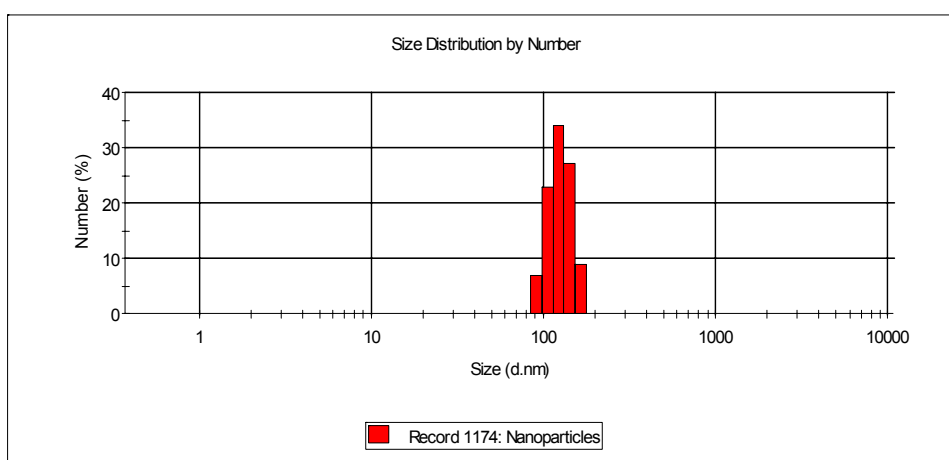
¹H NMR and DLS of **AbBD** nanoparticles prepared via the one-pot process.



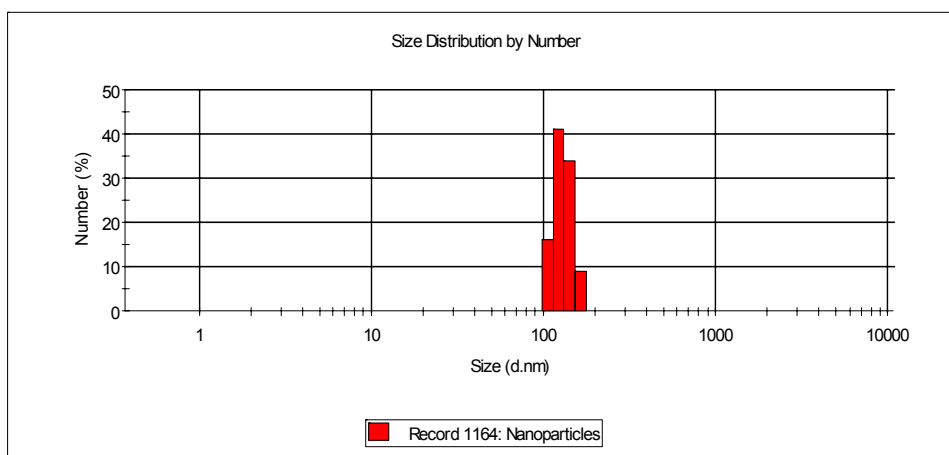
DLS size distribution profile for 120.5 ± 10.2 nm ABD-NP-HVGGSSV particles (**3**).



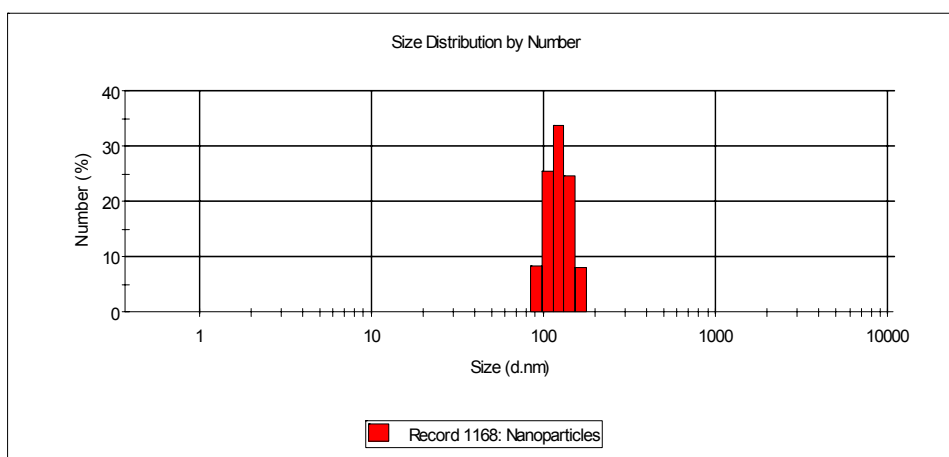
DLS size distribution profile for 129.4 ± 9.8 nm AbBD-NP-594-MT particles **(6)**.



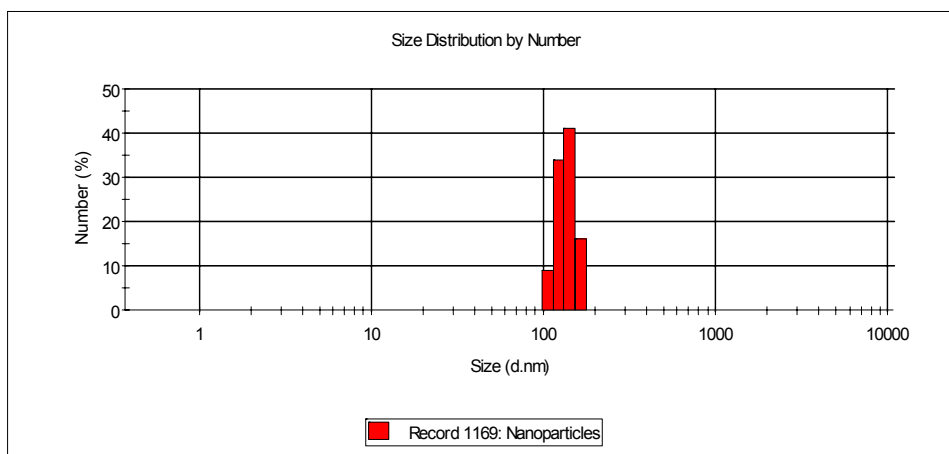
DLS size distribution profiles for 130.7 ± 9.4 nm AbBD-NP-cHVGGSSV-594-MT particles **(11)**.



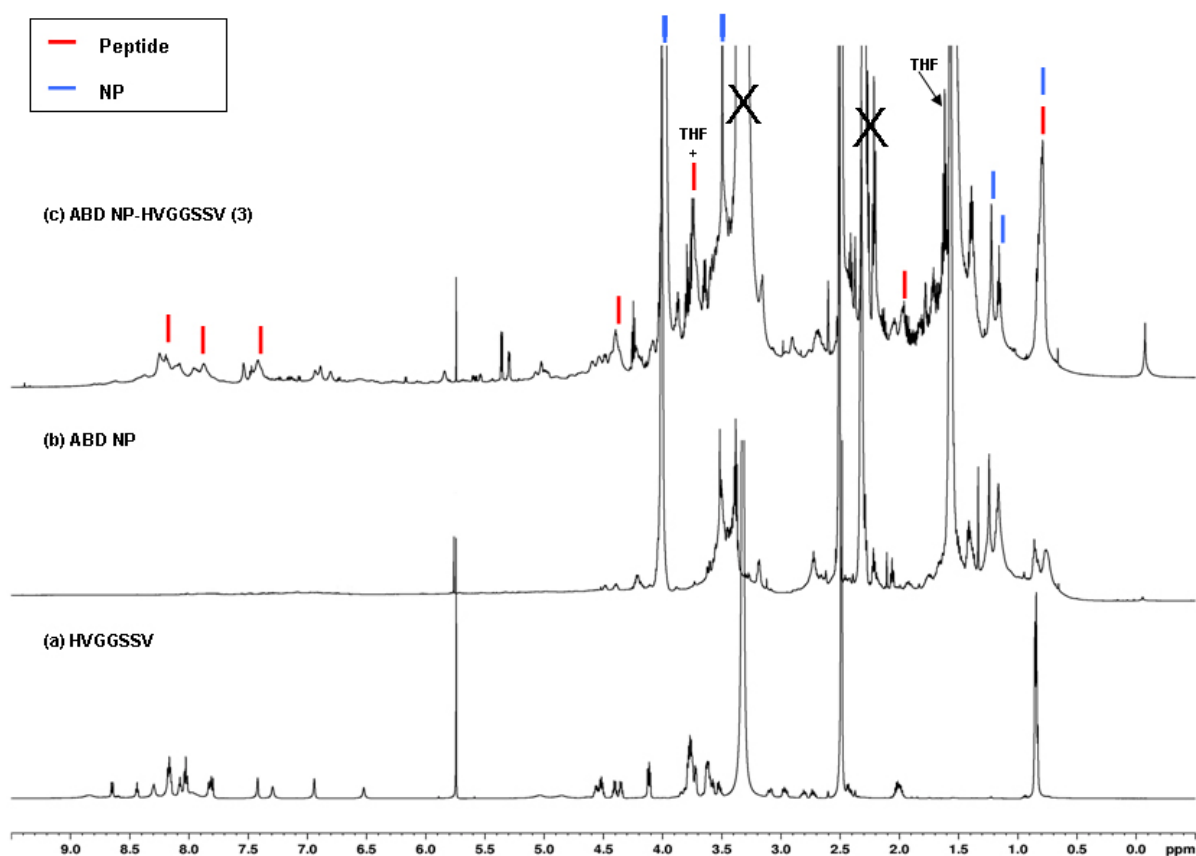
DLS size distribution profiles for 131.9 ± 10.6 nm AbBD-NP-594-cRGD-MT particles **(12)**.



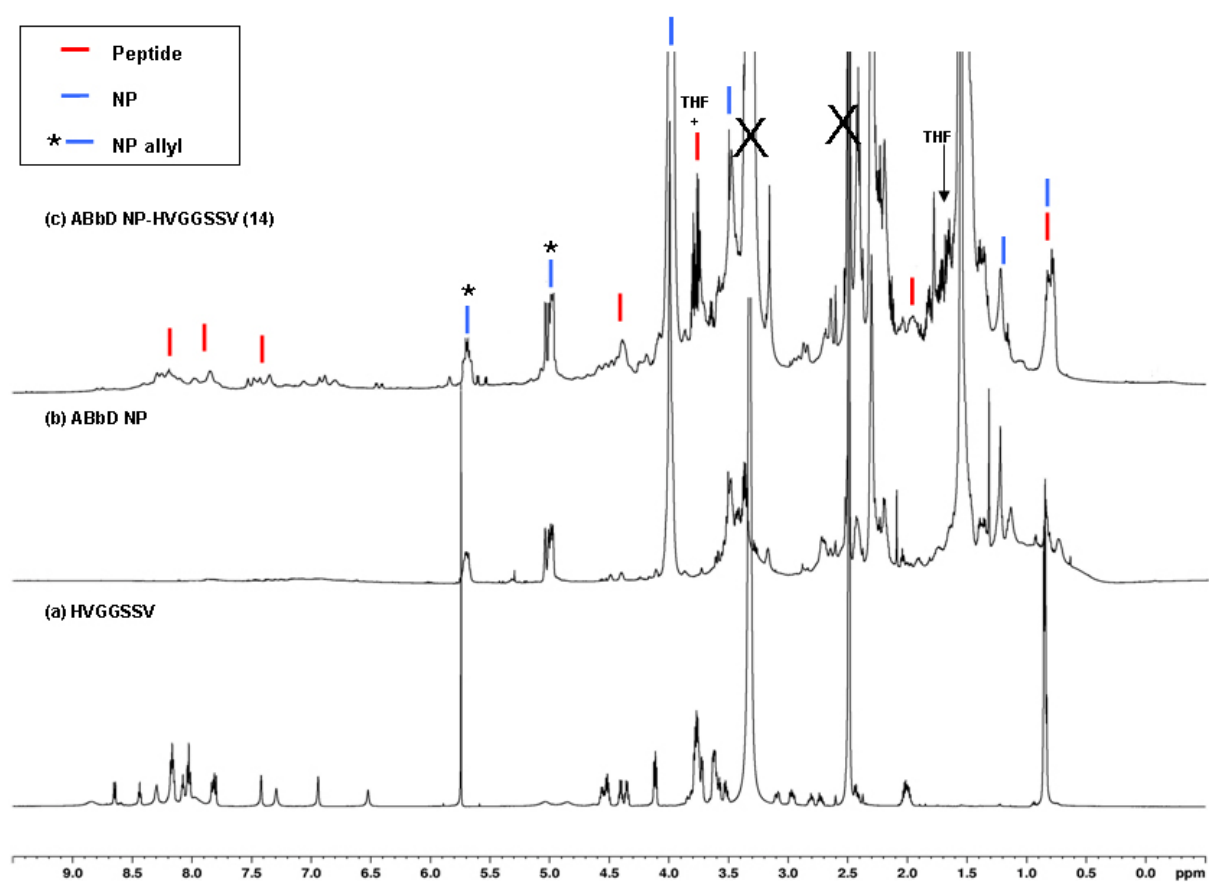
DLS size distribution profiles for 129.7 ± 9.5 nm AbBD-NP-HVGGSSV particles **(14)**.



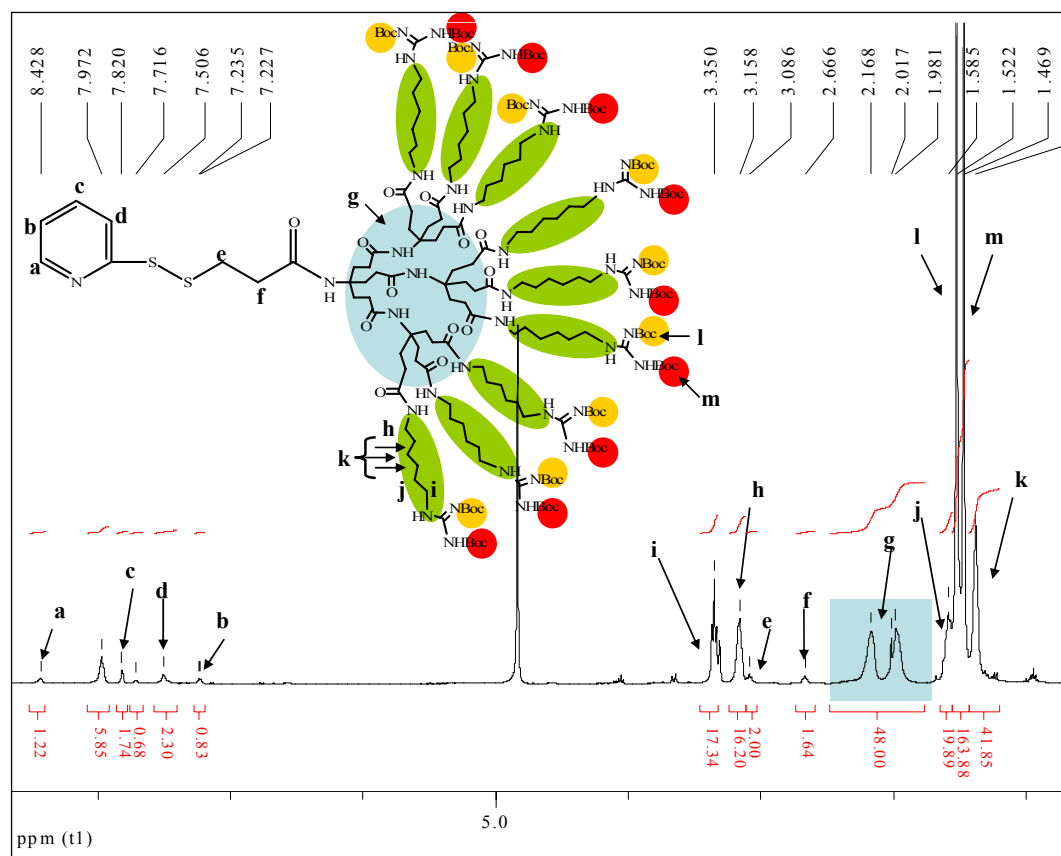
DLS size distribution profiles for 132.1 ± 9.3 nm AbBD-NP-HVGGSSV-594-MT particles (**16**).



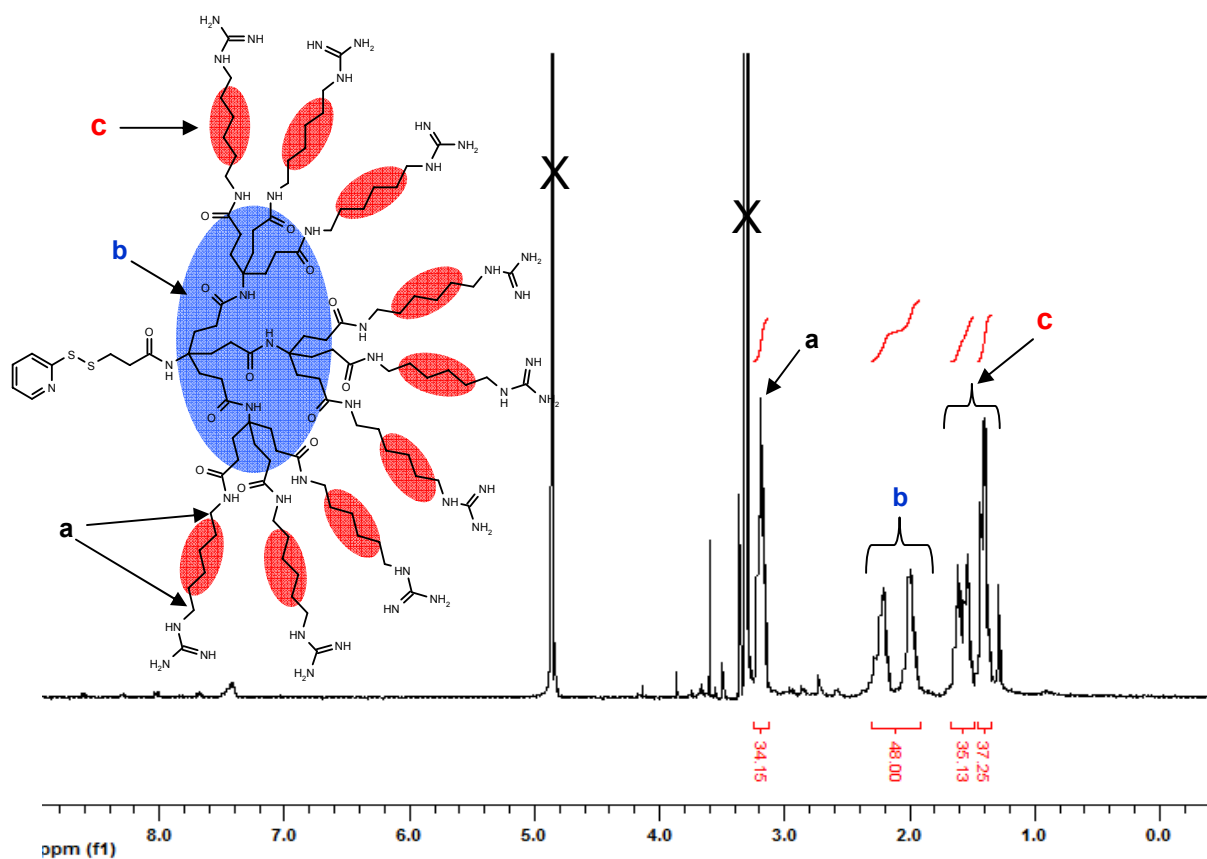
^1H NMR (600 MHz) spectra (a) GCGGGNHVGGSSV; (b) **ABD-NP**; (c) ABD-NP-HVGGSSV (**3**).



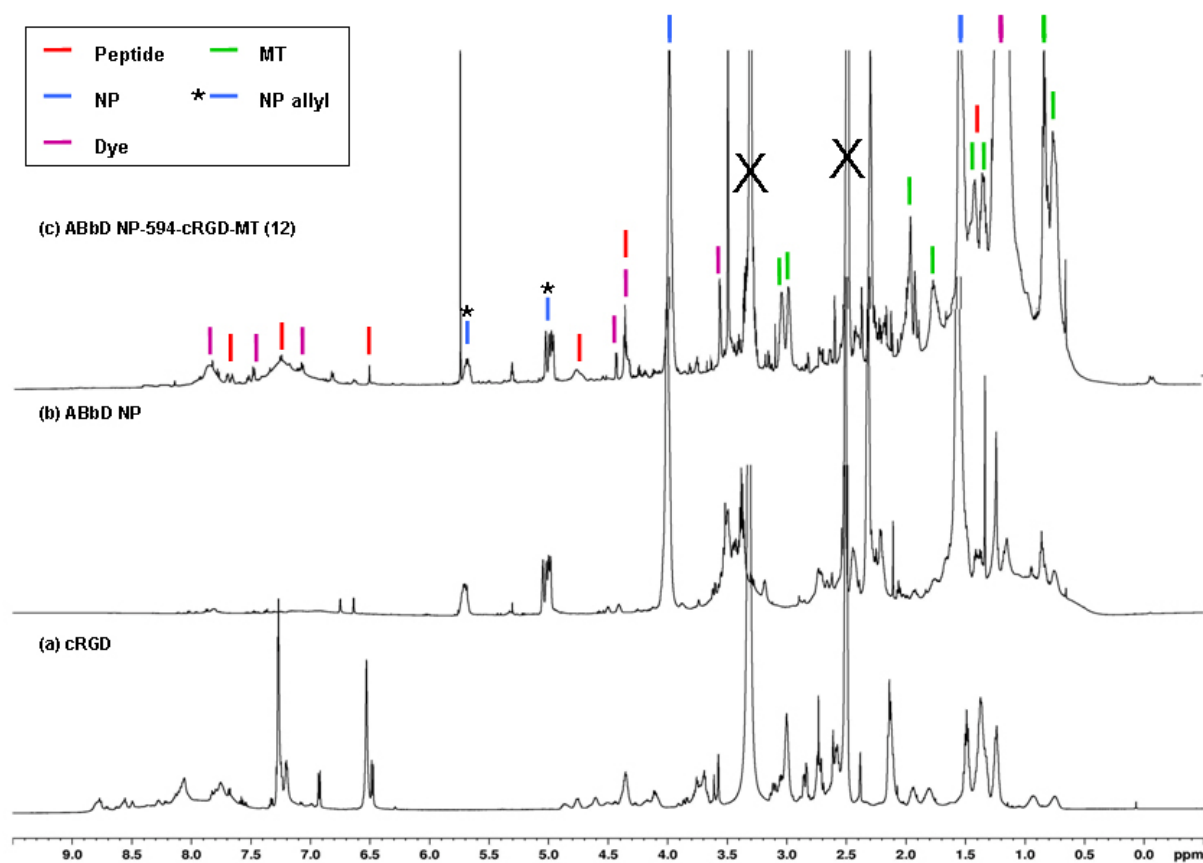
¹H NMR (600 MHz) spectra (a) GCGGGNHVGGSSV; (b) **AbBD**-NP; (c) AbBD-NP-HVGGSSV
(14).



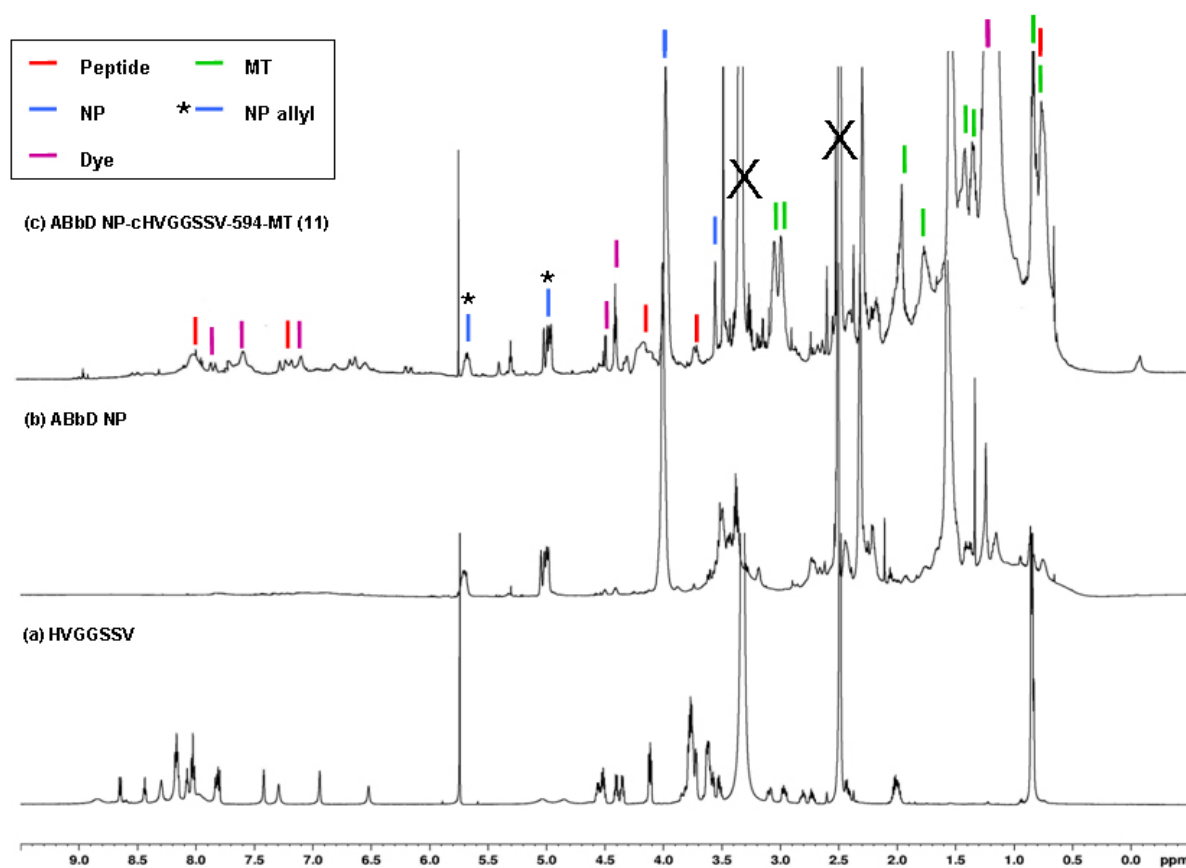
^1H NMR spectra of Boc protected MT in MeOH-d_4 .



^1H NMR spectra of Boc-protected MT in MeOH-d_4 prior to dithiol cleavage to give (5) (insoluble in DMSO-d_6).



¹H NMR (600 MHz) spectra (a) cRGD; (b) **AbBD**-NP; (c) AbBD-NP-594-cRGD-MT (**12**) in DMSO-d₆.



^1H NMR (600 MHz) spectra (a) GCGGGNHVGGSSV; (b) **AbBD**-NP; (c) AbBD-NP-cHVGGSSV-594-MT (**11**) in DMSO- d_6 .

NMR quantification of peptide or MT on nanoparticles utilizing the allyl groups via thiol-ene chemistry

The peak at 4.08 ppm is used as an inner standard to determine how many unmodified allyl groups are remaining which translates to how many peptides or MTs are attached to the particle

1. K. Huang, B. Voss, D. Kumar, H. E. Hamm and E. Harth, *Bioconjugate Chem.*, 2007, **18**, 403-409.
2. A. E. van der Ende, E. J. Kravitz and E. Harth, *J. Am. Chem. Soc.*, 2008, **130**, 8706-8713.